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Recent evidence suggests that the adenomatous polyposis coli (APC) gene participates in breast tumorigenesis. Although a precise biological function for APC protein has not yet been determined, it has been shown that the APC protein interacts with β-catenin and plakoglobin *in vivo*. β-catenin and plakoglobin are components of two specialized anchoring junctions, the adherens junction, a site of attachment for bundles of actin filaments, and the desmosome, a site of attachment for intermediate filaments (e.g. keratin). A direct correlation has been shown between loss of adherens junction components and the metastatic potential of breast cancer. I have used a combination of immunofluorescence microscopy and biochemical fractionation to determine the location of APC protein in epithelial cells from both normal and breast cancer tissue. APC protein located at the cell-cell junctions of MCF-7 cells co-localized with E-cadherin, β-catenin, and plakoglobin. Primary breast cells, deficient in APC protein did not form polarized structures when grown in matrigel. Mouse embryonic stem cells either heterozygous or homozygous for an APC mutation did not form embryoid bodies as efficiently as their normal parental counterparts. These findings are consistent with APC protein interacting with cell-cell junction proteins and effecting polarization.

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INTRODUCTION

Recent evidence suggests that the adenomatous polyposis coli (APC) gene participates in breast tumorigenesis. APC gene loss is reported in as many as 28% of human breast tumors (1). Female mice carrying mutant APC genes show an increased rate of breast hyperplasia and neoplasia (2). In addition, APC has been linked to the murine proto-oncogene Wnt-1, which was originally identified as a frequent target for insertional activation by mouse mammary tumor virus in mammary carcinomas. In vertebrates, APC protein is proposed to be a component of the Wnt-1 signaling pathway. Together, these data suggest a key role for APC in the breast cancer pathway.

Although a precise biological function for APC protein has not yet been determined, it has been shown that the APC protein interacts with β–catenin and plakoglobin in vivo (3, 4, 5). Like APC, β–catenin is a component of the Wnt-1 signaling pathway. In the absence of Wnt-1, the APC protein down-regulates β–catenin. If Wnt-1 is present, APC does not target β–catenin for degradation, thus allowing for its accumulation in the cytoplasm and subsequent transport to the nucleus where it can effect gene expression. β–catenin and plakoglobin are components of two specialized anchoring junctions. Anchoring junctions mechanically attach epithelial cells (and their cytoskeleton) to neighboring cells or to the extracellular matrix. β–catenin and plakoglobin are found in the adherens junction, a site of attachment for bundles of actin filaments. Plakoglobin is also a component of the desmosome, a site of attachment for intermediate filaments (e.g. keratin). Anchoring junctions also serve an important role in signal transduction, mediating changes in cyto-architecture and proliferation. A direct correlation has been shown between loss of adherens junction components and the metastatic potential of breast cancer. The purpose of this study is to determine whether APC interacts directly with adherens junctions or desmosomes through actin and keratin respectively. Demonstration of an interaction between APC protein and cell-cell junction components in normal cells would suggest that breast epithelial cells with mutant APC protein might have compromised junctions and therefore, decreased cell polarization. If APC protein can be demonstrated to be a component of the cell-cell junctions and thereby effect cell polarization, then this could explain the link between APC loss and breast cancer.

My research has demonstrated that breast epithelial cells containing only one wild-type copy of the APC gene have a severe deficiency in their ability to establish polarity when compared to normal breast-epithelial control cells. I am presently investigating the hypothesis that the APC protein plays an essential role in normal breast epithelial cell polarity and its absence contributes to the loss of cellular differentiation and growth regulation found in malignant transformation. In the second year of investigation, I have tested cells from human breast-cancer lines for localization and expression of APC protein, using both a biochemical and histological approach. I have analyzed co-localization of APC protein and cell-cell junction components E-cadherin, β-catenin, and plakoglobin. Finally, I have used primary breast epithelial cells and mouse embryonic stem cells to directly test the hypothesis that APC protein is required for cell polarization.

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BODY
EXPERIMENTAL METHODS
Cell lines and Tissue Culture. Cell lines used in these experiments were maintained at
37o C in CO2 (5%) incubators. 184A1 cells are an immortalized human mammary
epithelial cell line (a gift from Martha Stampfer). They were grown in MCDB 170 media
(Clonetics Corporation, La Jolla, CA) supplemented as described (6). Other cells were
obtained from the American Type Culture Collection (ATCC, Gainsburg, MD) and were
maintained in the following growth media: MCF7 [Eagle's Modified Essential Media
(MEM), 10% Fetal Bovine Serum (FBS), 1% nonessential amino acids, Earle's Basic Salt
Solution, 1 mM sodium pyruvate, 10 ug/ml insulin], MDA-MB468, MDA-MB221, and
HS578T [Dulbecco's MEM (DME), 10% FBS], BT549 and T47D [RPMI 1640, 10%
FBS]. Mouse embryonic stem cells (D3a2, C110, clone #54 and #64, generous gifts from
Mark Taketo) were maintained in an undifferentiated state by growth on feeder layers of
fibroblasts that have stable expression of GFP. Cells were fed media containing LIF in
order to inhibit their ability to differentiate.

Immunofluorescence microscopy. Cells were seeded onto tissue culture chamber slides
(25-50% confluency) and allowed to grow for 36 - 48 h before manipulation. Cells were
rinsed in Phosphate Buffered Saline (PBS) [10 mM phosphate, pH 7.5, 100 mM NaCl]
then fixed with 2% paraformaldehyde in PBS for 30 minutes (min) at 4o C. Following
two PBS rinses, cells were permeabilized with 0.2% tritonX-100 in Tris Buffered Saline
(TBS) [10 mM Tris, pH 7.5, 100 mM NaCl, 5 mM KCl] for 5 min at room temperature.
Following two TBS washes, cells were incubated with 0.5% Na_2BH_3 in water for 10 min
at room temperature. Cells were rinsed with TBS and then incubated with primary
antibody diluted in antibody buffer [1% Bovine Serum Albumin (BSA), 3% normal goat
sera, 0.2% tritonX-100 in TBS] for 90 min at room temperature. Cells were rinsed three
times with TBS prior to incubation with secondary antibody conjugated to Fluorescein-
isothiocyanate FITC, rhodamine or Texas Red for 30 min at room temperature. Cells
were rinsed three times with TBS and mounted with Pro Long antifade (Molecular
Probes, Eugene, OR) for immunofluorescence microscopy. Antibodies and dilutions
used for the experiments are as follows: APC (mouse IgG1, Ab-4) 1:150 (Oncogene
Science, Cambridge, MA), or APC (rabbit, APC64, a gift from Arnold Levine; or
APC270 produced locally) 1:100; α-tubulin (mouse IgG1, DM-1A) 1:200 (ICN
Biomedicals, Inc. Costa Mesa, CA), keratin (guinea pig, K-4252) 1:300 (Sigma Immuno
Chemicals, St. Louis, MO), lamin A/C (mouse IgG1, X-67) or lamin B (mouse IgG1,
X233) undiluted (American Research Products, Inc., Belmont, MA), β–catenin (mouse
IgG1, Transduction Labs) 1:1000, β–catenin N-termini (mouse IgG2a) 1:200, β–catenin
exon 3 (mouse) 1:1000), E-cadherin (mouse IgG1, Transduction Labs) 1:200), ZO-1
(mouse IgG1, Transduction Labs) 1:500, goat anti mouse IgG1-FITC and goat anti mouse
IgG1-Texas Red 1:200 (Southern Biotechnology Assoc. Inc., Birmingham, AL) and goat
anti rabbit-Texas Red 1:200 (Accurate Chemical and Scientific Corp, Westbury, NY),
and goat anti rabbit-FITC 1:200 (Boehringer Mannheim biochemicals, Indianapolis, IN).
An APC polyclonal serum was adsorbed to DLD-1 cells that had been fixed and
permeabilized as described for immunofluorescence staining. For APC antibody
blocking experiments, APC antibody was incubated with a peptide corresponding to APC
protein amino acids 2717 - 2844 at 10-fold molar excess in PBS for 12 hours at 4o C.
Any precipitant protein was pelleted by centrifugation for 15 min prior to dilution of the
peptide/antibody mixture in antibody buffer.

Immunoprecipitation. Cells were harvested in cold PBS as described and lysed by
sonication (2 x 30 seconds) in L-buffer. Insoluble proteins, membranes, and DNA were
removed by centrifugation 15,000 rpm, 15 minutes. Proteins in the supernatant were

incubated with 25 ul protein A sepharose beads 120 mg/ml (Sigma, St. Louis, MO) for 60 min, 4°C. Protein A Sepharose was removed along with cellular proteins that bind nonspecifically to it by centrifugation at 6500 RPM for 5 minutes. Primary antibody (2 mg) was incubated with the preadsorbed lysate for 60 minutes at 4°C on a rotator platform. Protein A sepharose (25 ul) was added to the antibody/lysate mixture and allowed to incubate for 1 hour at 4°C. Protein A Sepharose and primary antibody were removed, along with cellular proteins recognized by the primary antibody by centrifugation at 6500 RPM for 5 minutes. The supernatant from this centrifugation step was saved for SDS-PAGE. The pellet was washed two times with L-buffer and three times with RIPA buffer [50 mM Tris, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate (DOC), 0.1% SDS]. Final pellets were resuspended in 2X SDS-PAGE sample buffer and boiled for 5 minutes. Protein A Sepharose was removed by centrifugation at 6500 RPM for 5 minutes, prior to loading on a gradient gel for SDS-PAGE.

Western Immunoblot. Proteins (70 mg/lane; 35 mg/lane for scaffold fractions) were separated electrophoretically using 4-12% acrylamide gradient tris tricine gels (Novex, San Diego, CA) and Laemmli buffer. Gels were run for 2 hr at 125V with cold circulating water. Proteins were transferred to nitrocellulose (Schleicher and Schuel, Keene, NH) for 16 hours at 30V in transfer buffer [192 mM glycine, 20% Methanol, 25 mM tris base and 0.1% SDS] with circulating cold water. Rainbow molecular weight markers (Amersham, Arlington Heights, IL) were loaded in one lane of each gel for size standardization. Nitrocellulose membranes containing transferred proteins were blocked with 5% BSA in TBST [TBS, 0.1% tween 20] then incubated with primary antibody diluted in 0.5% BSA in TBST for 1 hr, 20o C. Following three, 10-min rinses with TBST, blots were incubated with a the appropriate secondary antibody conjugated to HRP in 0.5% BSA/TBST. Blots were rinsed three times with TBST and then probed using ECL chemiluminesence detection system (Amersham) as per manufacturer's instructions. Antibodies used for Western Immunoblot analysis were as follows: APC (mouse IgG1, Ab-1 or Ab-2) 1:500 (Oncogene Science), α-tubulin 1:200, α-adaptin (mouse IgG2a, AP-2) 1:200 (Sigma Immunochemicals), EGF-receptor (rabbit sera, Ab-4) 1:100 (Oncogene Science), lamins A, B, and C 1:10, HRP-Rabbit anti mouse IgG1 1:20,000 (Zymed Immunochemicals, South San Francisco, CA), horse radish peroxidase (HRP)-goat anti rabbit IgG and HRP-sheep anti mouse 1:40,000 (Sigma Immunochemicals),

Matrigel Cell Polarization Assay
Primary breast epithelial cells were prepared from reduction mammoplasties and needle biopsy from and FAP patient and subjected to matrigel cell polarization assay as described (Petersen *et al).* Briefly, after disaggregation and culture of primary specimens, cells were passaged into 300 ul EHS Matrix (10mg/ml, obtained as Matrigel; Collaborative Research) as single cells at 2.5 x 10e5 cells per well of a 4-well plate (Nunc) The medium was changed every third day at which time cultures were monitored for structure formation.

Embryoid body formation and Tight Junction Assay
For embryoid body formation, 6 x 10e6 cells, maintained on fibroblast feeder layers, were trypsinized and cultured on gelatin-coated 6-well dishes for 3 d in 90% DMEM/10% FBS, and then subjected to suspension culture in three 10-cm bacterial dishes. Medium was changed every other day. Tight junction permeability assays were performed as described by Saiton et al. (7). Wild-type and APC-deficient cystic embryoid bodies were incubated with NHS-LC-biotin (1mg/ml, Pierce Chemical Co.) in HBS (+) [HBS containing 1 mM Ca Cl2 and 1 mM Mg Cl2] for 30 min. After washing

with HBS (+) three times, embryoid bodies were fixed with 1% paraformaldehyde in PBS, pH 7.4 for 1 h at room temperature. Fixed samples were frozen in isopentane cooled with liquid nitrogen, and sectioned on a cryostat at a thickness of 12 um. After 1 hr rinse with PBS/ 1% BSA, cells were incubated with rhodamine-phalloidin (1:200) and FITC-conjugated ExtraAvidin (1:200, Pierce). Samples were mounted and viewed as for immunofluorescence microscopy.

RESULTS

Location of APC protein in breast epithelial cells from normal and cancer cell lines as determined by imunofluorescence microscopy.

Task 1: Plate cells from breast cancer lines established in the lab and purchased from ATCC on glass chamber slides. Test cells for wild-type APC protein expression by immunofluorescence analysis. Characterize localization of APC protein in the cancer lines grown on a slide.

The distribution pattern for endogenous, full-length APC protein in various cell lines was determined using indirect immunofluorescence microscopy with antibodies that recognize the C-terminus of APC protein. The staining pattern in four breast cancer cell lines, BT549, MDA-MB468, MCF7 and T47D, was compared to those seen in cells from primary outgrowth of normal breast epithelial tissue (BE20, BE-21) and the "normal" breast cell line 184A1.

Cells from the four breast cancer lines also showed a similar staining pattern to that seen in 184A1 cells. In each case, cells were stained using antibodies directed against the C-terminus of APC protein (monoclonal Ab-4 and polyclonal sera, APC64) and IgG1 as a negative control. T47D (Fig. 1j) and MDA-MB468 (Fig. 2a) cells displayed both punctate cytoplasmic staining, concentrated at the leading edge, and nuclear staining. A similar pattern was seen in BT549 cells, with additional filamentous staining visible in the cytoplasm (Fig. 2b). Non-confluent MCF7 cells also displayed the punctate cytoplasmic and nuclear staining pattern (Fig. 2c). However, in areas of higher cell confluency, the staining pattern was strikingly different. While the nuclear staining was still clearly visible, a significant portion of the APC protein appeared to be located at cell-cell junctions (Fig. 2d).

Since APC protein was found concentrated in regions of cell-cell contact, I was interested if this APC protein co-localized with various components of adherens junctions, E-cadherin, β–catenin and plakoglobin. Such overlap in localization pattern might suggest that these different proteins interact directly or are in a large complex at the adherens junction.

As shown in figure 3, APC (3a) and E-cadherin (3b) have overlapping localization patterns. Similarly, APC (3d) and plakoglobin (3e) proteins co-localize. Colocalization between APC and β–catenin proteins was shown using combinations of three different APC antibodies; Ab-4 (3g), APC64 (3j) and APC270 (3m, 3p) and three different β–catenin antibodies; β–catenin N-termini (3h), β–catenin (3k, 3n) and β–catenin exon 3 (3q).

Western Immunoblot analysis of APC protein in breast cancer cell lines.

Task 2: Test cells found to have no detectable level of APC protein in Task 1 for APC expression by Western immunoblot analysis. Determine percentage of breast cancer lines that have decreased APC protein compared to normal.

In the colon, >99% of the inactivating APC mutations result in production of a truncated APC protein product, thus implying that point mutations are not sufficient to inactivate the very large APC protein. In addition, surveys of colon polyps (colon cancer precursors) show that the vast majority has inactivated both copies of the APC gene (8). Assuming that the same is true for epithelial cells of the breast, it is possible to catalogue the percentage of breast cancer cell lines with fully-inactivated APC by testing these lines for the presence of full-length APC protein.

In order to confirm the presence of full-length APC protein, lysate from one additional breast cancer cell line, MCF-7 cells was tested for APC expression by Western blot analysis. Lysates from MCF-7 contained a 320 kDa protein that was recognized by an antibody raised against APC protein's N-terminus (Fig. 4). Based on the combined immunofluorescence and Western immunoblot data, I conclude that five out of five breast cancer cell lines tested express full-length APC protein.

Polarization of APC-deficient cells

Task 3: Test cells found to have no detectable level of APC protein in Task 1 for ability to polarize.

Since all five breast cancer cell lines tested appear to have normal APC protein, I initially examined polarization in primary breast epithelial cell outgrowth from breast tissue heterozygous for mutant APC. The cell polarization assay took advantage of a matrigel 3-Dimension system, technology that has recently been introduced in our lab. Normal breast epithelial cells plated in matrigel will form structures within 10 days (7). These structures are made up of a sphere of approximately eight polarized cells, with apical regions facing a central, fluid-filled lumen. In contrast to normal breast epithelial cells, primary outgrowth from patients heterozygous for mutant APC (+/-), did not form structures in matrigel, even after 29 days and remained as single cells (Fig. 5). Lack of structure formation was not due to senescence of the cells, since APC (+/-) cells passed at the same time but plated on plastic directly, rather than in matrigel survived and proliferated. Inability to form structures was also not due to lack of the right "type" of breast epithelial cell in the APC (+/-) culture. Structures are formed from cells expressing keratin 18 (K18), the luminal epithelial cells (7). Fluorescence Activated Cell Sorting (FACS) analysis with antibodies to K14 and K18 demonstrated that 86 % of the APC (+/-) epithelial cells were K18 positive, and thus, should have been competent to form structures (data not shown).

Creation of "null" breast epithelial cells for use in polarization assay - Task 4

Since all five breast cancer cell lines tested appear to have normal APC protein, it was necessary to create an APC "null" cell line in order to fully test potential APC functions. Several methods were employed, each based on the ability of an antisense oligonucleotide to block translation of the APC protein. The first method involved design and synthesis of DNA oligomers, 18 nucleotides long, that corresponded to sequences "antisense" to APC mRNA, either in the 5' untranslated region (APC-A5) or spanning the initiation codon (APC-AC) . "Sense" oligos were synthesized in parallel for use as negative controls (APC-S5 and APC-SC). The oligos were purified by HPLC, dialyzed, then added to the growth media of 184A1 cells, both in the presence and absence of a lipofection reagent. Lipofection has been reported to improve permeability of DNA oligos into some types of cells. Although a range of oligo concentrations was tested, using two different antisense oligos, I was unable to detect changes in APC protein level as determined by immunofluorescence analysis (data not shown).

For the second antisense approach, the entire APC gene was cloned in reverse orientation into the expression vector pCDNA3.1 (Invitrogen). Cells were co-transfected with the antisense APC construct and a green fluorescent protein- (GFP) expression construct so that APC protein level could be monitored specifically in those cells that were successfully transfected. Although GFP-expressing cells could be identified after co-transfection, no reduction in APC protein level could be detected by immunofluorescence analysis.

The third antisense approach utilizes the retroviral construct pLXSN (Clontech) containing the complete cDNA for APC in reverse orientation. Transfection of a colon cancer cell line that contained no endogenous full-length APC protein with this construct did not result in alteration of cellular morphology.

Based on the difficulties producing an APC null breast epithelial cell line, I chose to examine polarity phenotypes in a different system, the mouse embryonic stem cell. Polarization of mouse embryonic stem cells with normal APC protein was compared to polarization in cells lacking normal APC protein. When normal mouse embryonic stem cells are allowed to differentiate for four days in suspension, they can form simple embryoid bodies. These embryoid bodies are comprised of a sphere of polarized cells, sometimes fluid-filled. The simple embryoid body continues to develop into cystic embryoid bodies by day 8. This structure is at least 2 cell layers thick, with an outermost layer of large endodermal cells and an inner layer of ectodermal cells. Such structures were allowed to develop from all 4 ES cell lines. At three days post suspension, the parental ES cell line (APC +/+) formed simple embryoid bodies (figure 6a). The ES cells heterozygous or homozygous for mutant APC (APC +/- line, 6b, and the APC -/-, 6c and d) formed at least ten-fold fewer structures than the parental line. These structures often looked more like small cell aggregates than actual embryoid bodies. I am presently analyzing the embryoid bodies from the parental (APC +/+) and the other APC deficient ES cell lines for tight junction formation.

APC Interactions with Intermediate Filament Proteins

Task 9: Precipitate keratin proteins from normal HMEC lysate. Test for co-precipitation of APC protein

In order to demonstrate interaction between APC protein and filament protein keratin, I have tested for co-immunoprecipitation. These experiments have been performed by precipitation with APC antibodies followed by Western immunoblot with keratin antibodies as well as the converse. Although I was able to demonstrate co-precipitation of APC and β–catenin protein using this method, and was able to show immunoprecipitation of keratin protein from a soluble cytoplasmic fraction, I was unable to clearly show a specific interaction between APC protein and keratin (data not shown). This might be because keratin is in a large complex, which masks the epitope, recognized by the precipitating antibodies. Similarly, APC antibody might only be able to precipitate soluble forms of APC protein that are in complex with only a few other proteins (such as β–catenin). We are currently testing various cell lysis/solubilization methods to overcome this difficulty. Alternatively, the interaction might be only transitory or weak.

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Discussion and Recommendations in relation to the Statement of Work:

Endogenous APC protein has been found in both the nucleus and the cytoplasm of cultured human breast epithelial cells. Immunocytochemistry revealed a particulate distribution of APC protein in distinct nuclear regions, and throughout the cytoplasm, with concentrations at the leading edge of migrating cells. Each of three monoclonal, and one polyclonal, antibodies directed against APC protein's C-terminus consistently showed the same staining pattern. In contrast, five antibodies directed against the APC protein's N-terminus failed to show significant staining, suggesting that the N-terminal epitopes might be masked in vivo, or that these antibodies might recognize denatured but not native protein.

Surprisingly, all five breast cancer cell lines tested expressed full-length APC protein. The distribution of APC protein in cells from these lines was remarkably similar to that in normal breast cells. The MCF-7 cell line revealed an additional location for APC protein, the cell-cell junction. Further characterization of this APC pattern in confluent MCF-7 cells revealed that the APC protein appearing at the cell-cell junctions is co-localized with known components of the adherens junction β–catenin, E-cadherin, and plakoglobin.

Since none of the breast cancer cell lines tested lacked full-length APC protein, I have used APC (+/-) breast cells from primary tissue for polarization studies. Initial results indicate that, while normal breast epithelial cells will form polarized cell structures when grown on matrigel, whereas the APC (+/-) cells do not. We are currently repeating this experiment and expanding the sample size. In addition, we are using embryoid body formation of APC (+/+), (+/-) and (-/-) embryonic stem cells as a second method to measure cell polarization ability.

CONCLUSIONS
    1. APC protein located at the cell-cell junctions of confluent MCF-7 breast epithelial cells co-localizes with E-cadherin, β–catenin and plakoglobin.
    2. Primary breast epithelial cell outgrowth from tissue heterozygous for an APC mutation does not form polarized structures when grown in matrigel.
    3. Simple embryoid bodies form at a much lower efficiency from mouse ES cells lacking normal APC (APC -/- and APC +/-) than from the normal parental lines.

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APPENDIX

Figure 1. Localization of APC protein in 184A1 cells using immunofluorescence microscopy.

184A1 cells were grown on glass slides prior to fixation and immunofluorescence microscopy using Ab-4, an antibody specific for APC protein (b, c) or using Ab-4 preincubated with an APC peptide (e). (b) and (c) are photographs of the same group of cells taken at two focal distances to more clearly capture cell edge staining (b, solid arrow) and nuclear staining (c, open arrows). APC protein appears in a punctate pattern throughout the cytoplasm with areas of protein concentration at one edge (solid arrows). In addition, APC protein appears throughout the nuclei with a few areas of concentration (c). (a) and (d) are differential interference contrast (DIC) and DAPI views of the fluorescence views shown in (b and c), and (e), respectively. Controls include: f, 184A1 cells stained with nonspecific antibody IgG1; g-i, 184A1 cells stained for APC using antibodies Ab-2 (g), Ab-6 (h) or APC64 (i); j, APC staining of breast cancer cell line T47D cells. For each antibody, both edge staining (solid arrows) and nuclear staining (open arrows) are apparent. In k and l, DLD-1 cells, which express only truncated APC protein, were stained using the C-terminal antibody Ab-4 to demonstrate staining specificity. Bar, 10 mm.

	DIC/DAPI	edge focus	nuclear focus
APC Ab-4	a	b	c
APC Ab-4 + block	d	e	f (IgG₁)
APC	g (Ab-2)	h (Ab-6)	i (APC64)

184A1

| APC Ab-4 | j (T47D) | k | l |

DLD-1

15
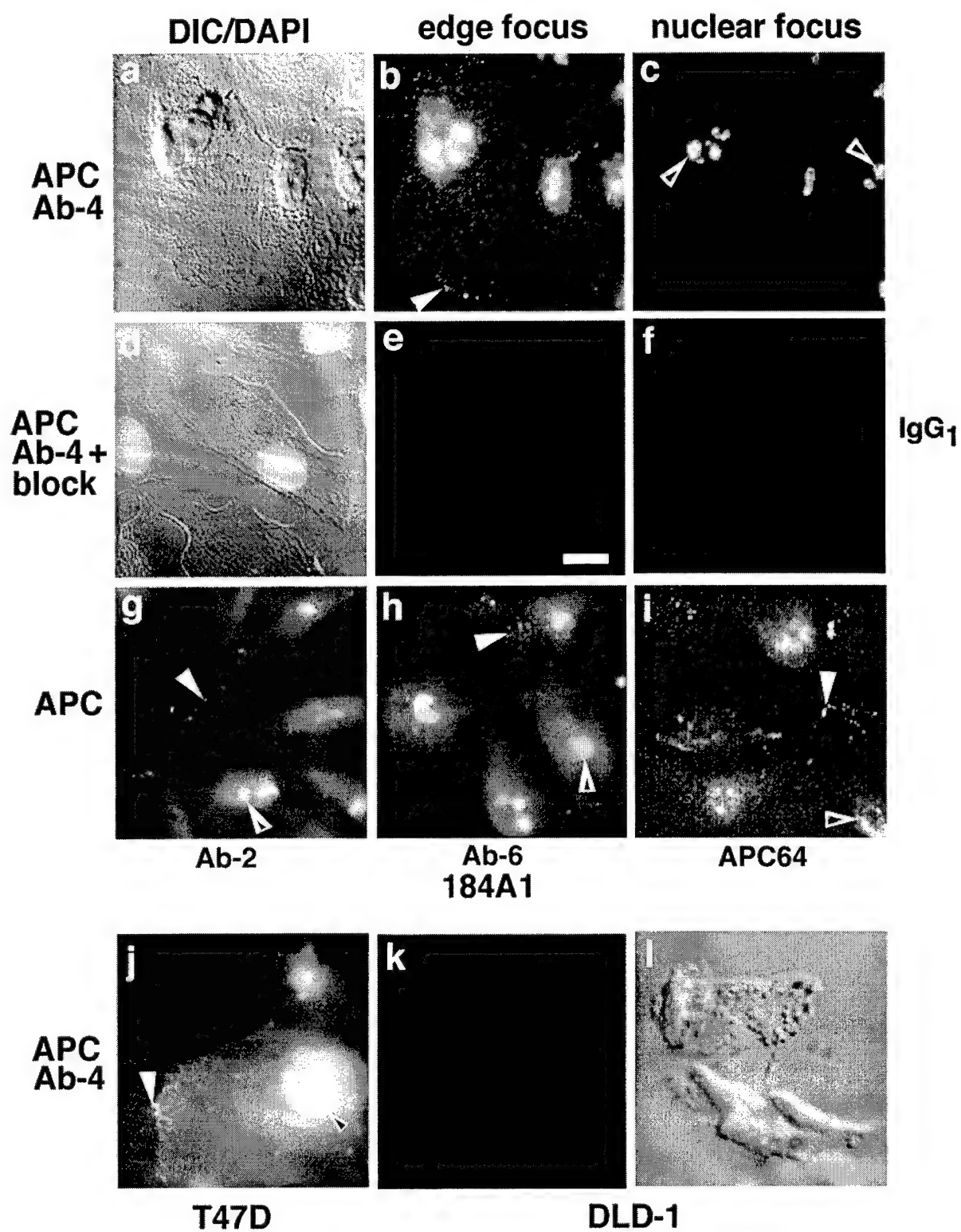
Figure 2. Localization of APC protein in cells from breast cancer cell lines.

Cells were grown on glass slides and stained with APC antibodies as described. The APC staining patterns of three breast cancer cell lines, not grown to confluence, MDA-MB468 (a), BT549 (b), and MCF-7 (c) are shown. As in 184A1 cells, the APC protein appears in a punctate pattern throughout the cytoplasm with areas of protein concentration at one edge, and also, throughout the nuclei with a few areas of concentration. In (d), MCF-7 cells were allowed to grow to confluence prior to staining, and display APC protein at cell-cell junctions.
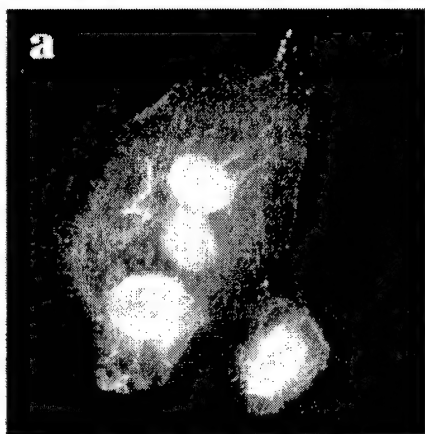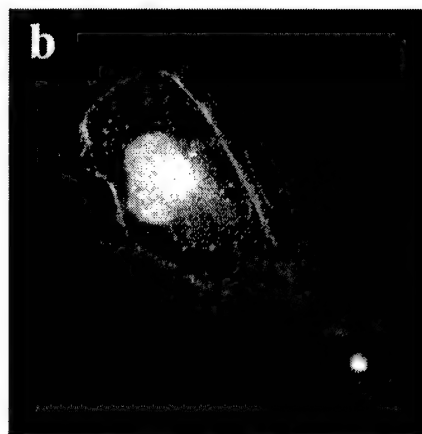
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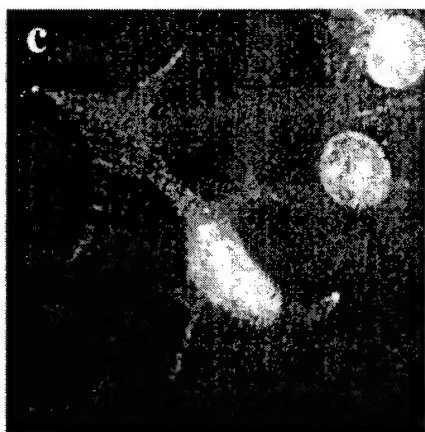
MCF7
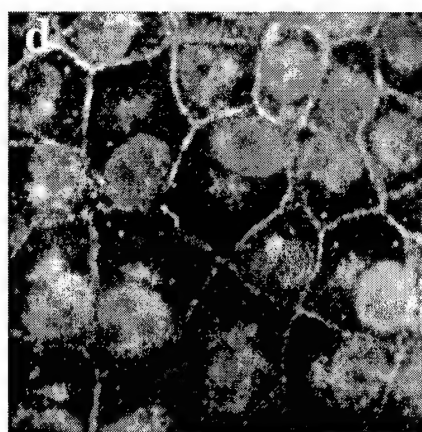
Figure 3 APC Protein localization pattern overlaps that of cell-cell junction proteins E-cadherin, β–catenin, and plakoglobin.

MCF-7 cells were grown to confluency on glass slides and were then stained for APC protein (left column) and a cell-cell junction protein (middle column). DAPI counterstained nuclei are shown in the right column. Antibodies used for staining were APC Ab-4 (a), E-cadherin (b), APC Ab-4 (d), plakoglobin (e), APC Ab-4 (g), β–catenin N-termini (h), APC64 (j), β–catenin (k), APC270 (m), β–catenin (n), APC270 (p) and β–catenin exon 3 (q).

APC Protein　　Junction Protein　　DAPI (nuclei)
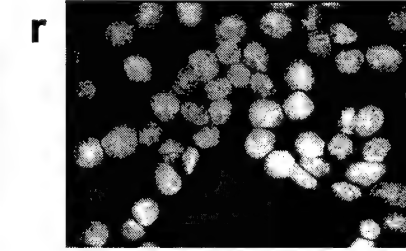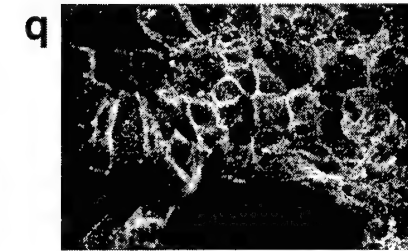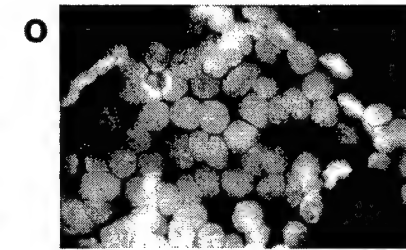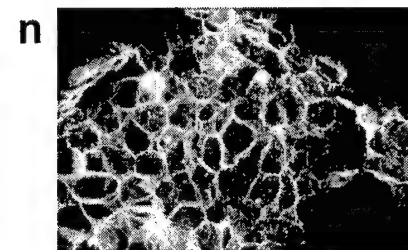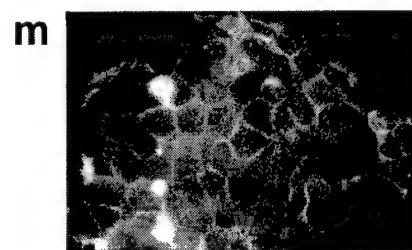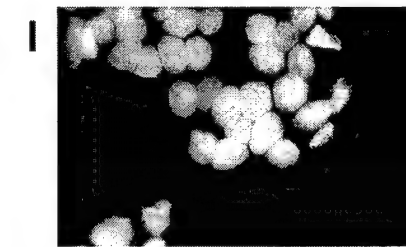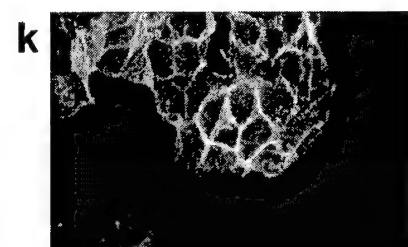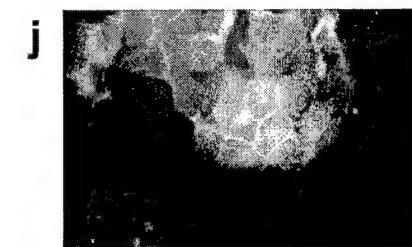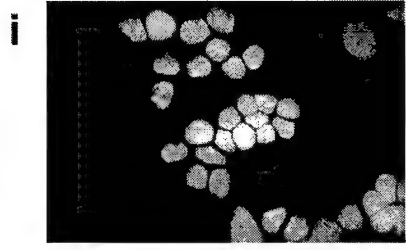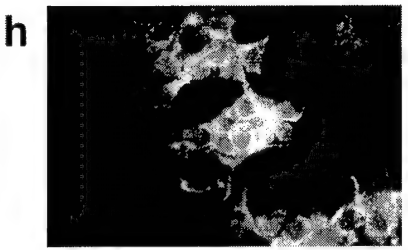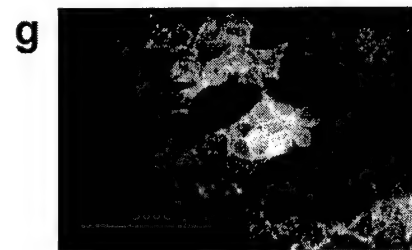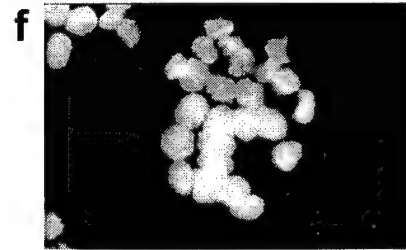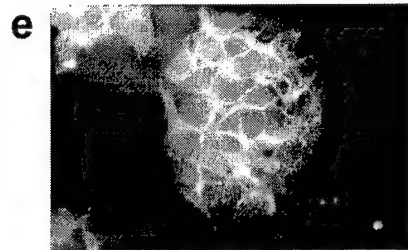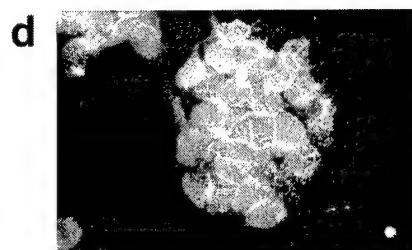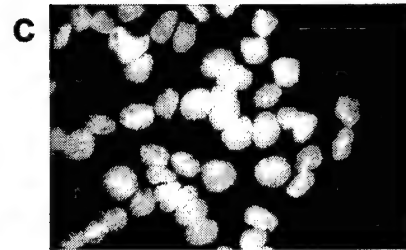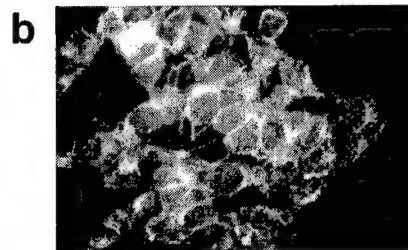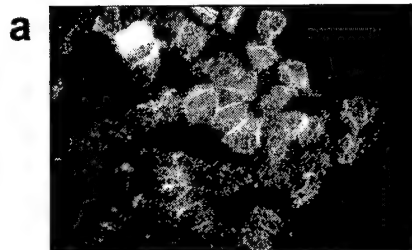
Figure 4. MCF-7 cells have full-length APC protein.

MCF-7 cells were lysed and subjected to electrophoretic protein separation and Western blot analysis. Using an antibody against the N-terminus of APC, full-length APC protein is seen as a clearly visible band, migrating above the 220 kDa molecular weight marker.
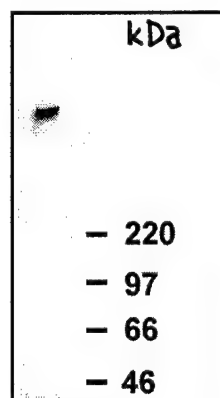
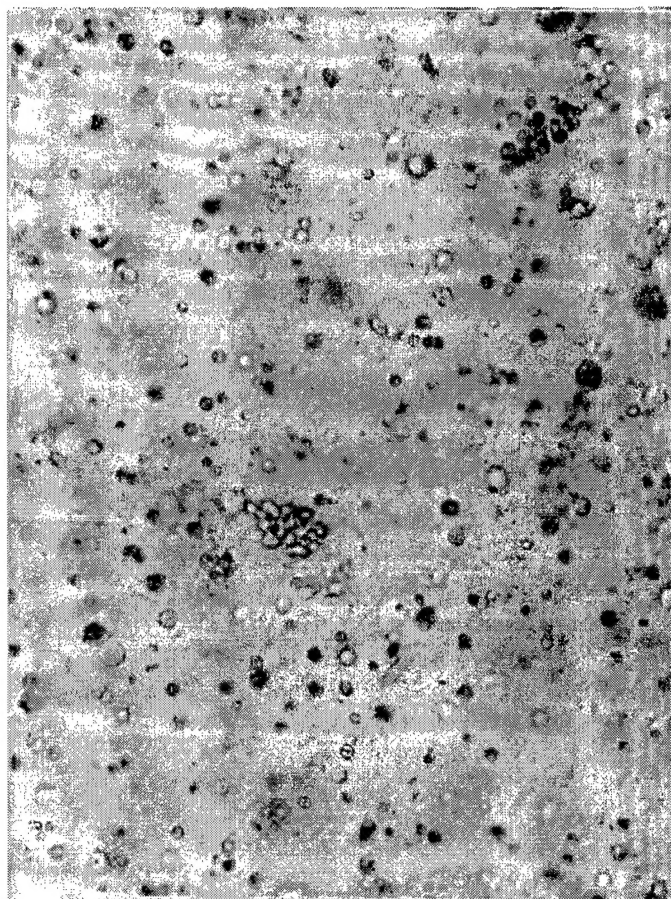Figure 5. APC (+/-) cells do not form polarized structures when plated in matrigel.

Breast epithelial cells from primary outgrowth of APC (+/-) tissue were seeded in matrigel and allowed to form structures. Cells had not formed structures by 11 days (a) or by 29 days (b).
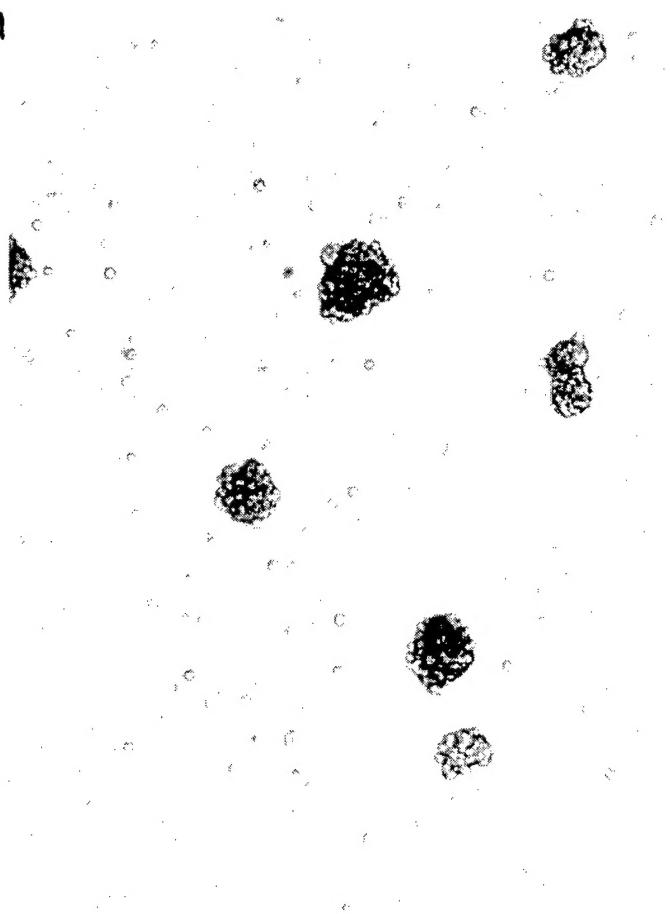
d 11 (16.5x)

d 29 (6.3x)

Figure 6. APC (+/-) and (-/-) embryonic stem cells are deficient in formation of simple embryoid bodies
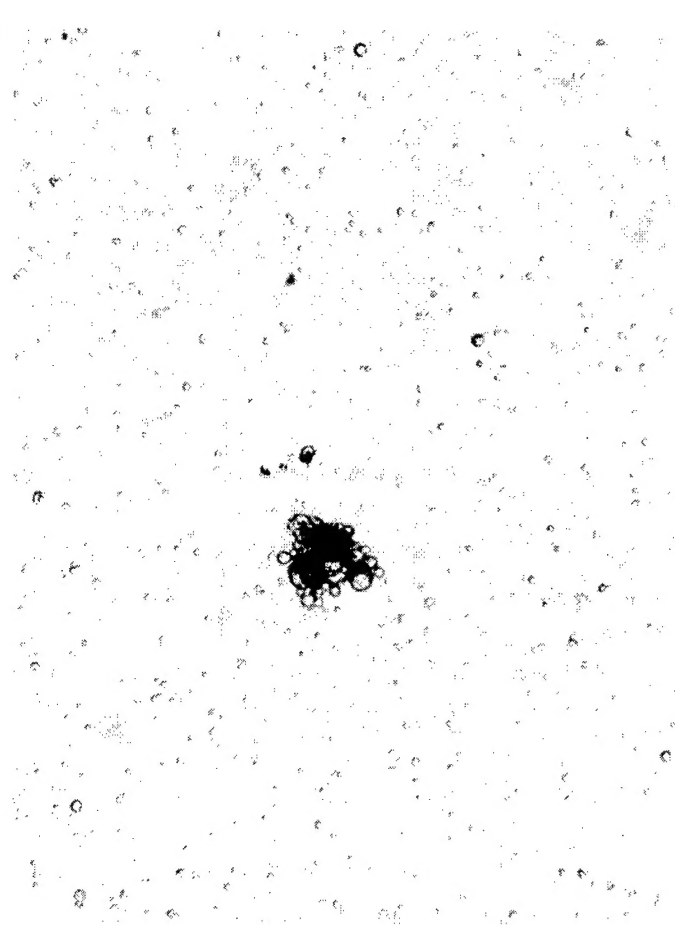
Cells from ES lines with normal APC (+/+) (a), deficient in APC (-/-) (c, d) or heterozygous for an APC mutation (+/-) (b) were allowed to form embryoid bodies. Only the parental cell line was able to efficiently form a simple embryoid body by day 4.
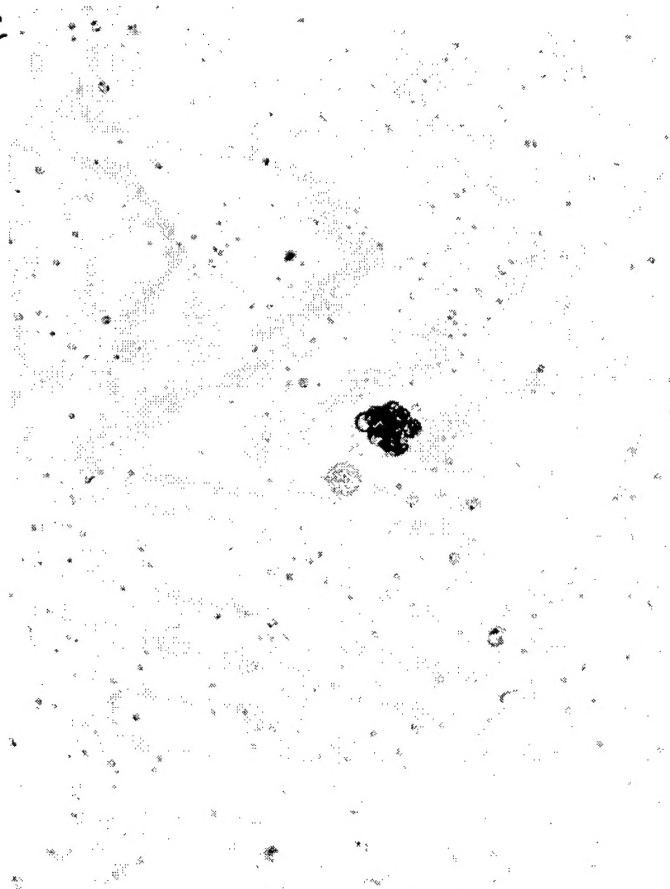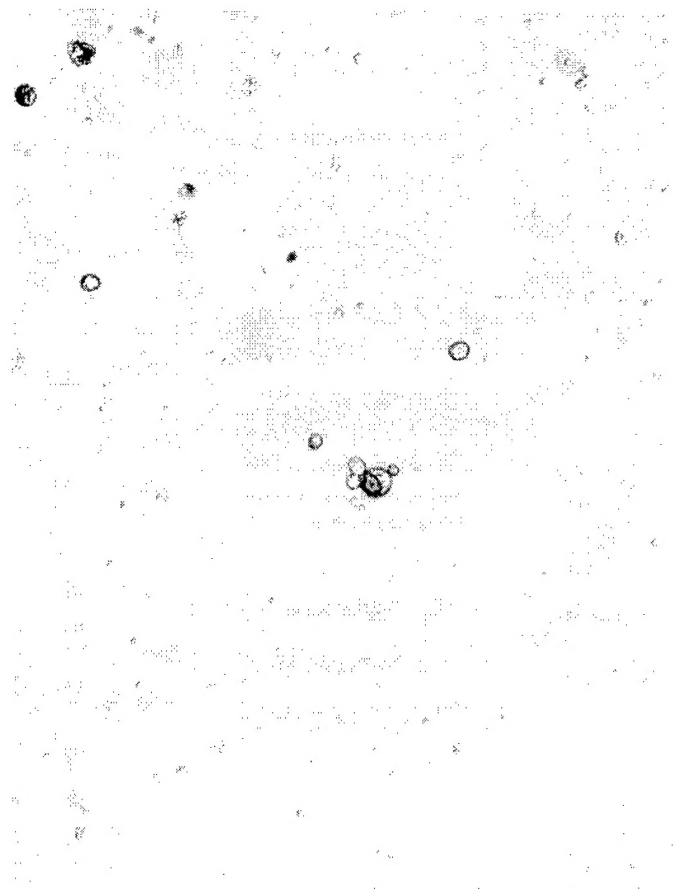
a

APC +/+

b

APC +/-

c

APC -/-

d

APC -/-